Supplementary material

Lowering Lipophilicity by Adding Carbon:

AzaSpiroHeptanes – A log *D* Lowering Twist

Sébastien L. Degorce, Michael S. Bodnarchuk and James S. Scott.*

Medicinal Chemistry, Oncology, IMED Biotech Unit, AstraZeneca, Cambridge Science Park, Unit 310 Darwin Building, Cambridge CB4 0WG, United Kingdom.

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***N*-[*Trans*-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)cyclohexyl]-5-(tetrahydro-2H-pyran-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine 5b** 4

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Synthesis

All solvents and chemicals used were reagent grade. Anhydrous solvents THF, DCM, and DMF were purchased from Aldrich. Flash column chromatography was carried out using prepacked silica cartridges (from 4 g up to 330 g) from Grace, Redisep or Silicycle and eluted using an Isco Companion system. Purity and characterization of compounds were established by a combination of liquid chromatography−mass spectroscopy (LC-MS) and NMR analytical techniques and purity was >95% for all test compounds.

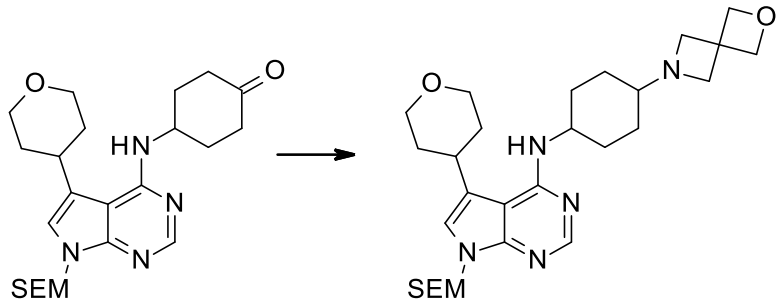
^{1}H NMR were recorded on a Bruker Avance 500 (500 MHz) or Bruker Avance DPX400 (400 MHz) and were determined DMSO-d_6. Chemical shifts are reported in ppm relative to TMS (0.00 ppm) or solvent peaks as the internal reference. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak.

Analytical LCMS was carried out using a suitable system, such as a Waters 2790/95 LC system with a 2996 PDA and a 2000 amu ZQ single quadrupole mass spectrometer, or a UPLC system utilising a Waters Aquity Binary pump with sample manager, Aquity PDA and SQD Mass spectrometer.

Accurate mass and MSMS fragmentation data were obtained using a Thermo Scientific hybrid LTQ-FT Mass Spectrometer with an Agilent 1100 Quaternary pump with PDA and Autosampler; 5µL of sample dissolved in 50:50 acetonitrile:water 0.1% formic acid was injected onto a Thermo Scientific Hypersil Gold 50 x 2.1mm 5µm particle LC Column and eluted with a gradient of 5 to 100% B over 17 min with 3 min re-equilibration time at 5% B. The flow rate was 0.5 mL/min with A being 0.1% formic acid in water and B 0.1% formic acid in acetonitrile. The MS and MSMS spectra were obtained in ESI +ve mode in both the ion trap and Ion Cyclotron Resonance (ICR) cell using helium as the collision gas at a normalized collision energy of 35eV. The ICR cell was run at resolution settings of 25000 in MS mode and 12500 in MSMS mode.

The syntheses of molecules **5b** and **21b** follow the same reported procedures as previous publications by our group for similar compounds.[1-3]

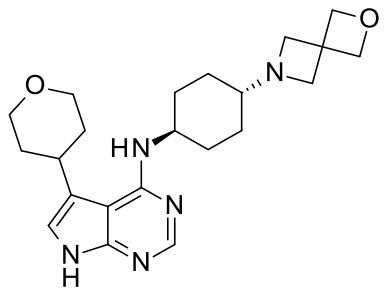
***N*-[4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)cyclohexyl]-5-(tetrahydro-2H-pyran-4-yl)-7-([2-(trimethylsilyl)ethoxy]methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine**



AcOH (0.10 mL, 1.8 mmol) was added to a solution of 4-((5-(tetrahydro-2H-pyran-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)cyclohexanone (400 mg, 0.9 mmol) and 2-oxa-6-azaspiro[3.3]heptane, 0.5 Oxalic acid (143 mg, 0.99 mmol) in MeCN (10 mL) at 25 °C. The resulting mixture was stirred at rom temperature for 3 hours. Sodium triacetoxyborohydride (381 mg, 1.80 mmol) was then added, and the suspension stirred for another 16 h, after which the reaction mixture was filtered The crude product was purified by flash C18-flash chromatography, elution gradient 5 to 80% MeOH in (0.1% HCO_2H) water. Pure fractions were evaporated to dryness to afford the title compound (racemate, 370 mg, 78%) as a colourless solid.

m/z (ES^+), $[M+H]^+$ = 528.6. ^1H NMR (400 MHz, DMSO-*d6*, 300 K) –0.09 (9H, s), 0.80 (2H, td), 1.08 (1H, q), 1.35 - 1.64 (7H, m, overlapped), 1.70 - 1.90 (4H, m), 1.92 - 2.02 (1H, m), 2.26 (1H, s), 3.43 - 3.62 (7H, m, overlapped), 3.87 - 3.96 (2H, m), 3.97 - 4.21 (1H, m), 4.62 (4H, d), 5.44 (2H, s), 5.79 (1H, dd), 7.03 (1H, d), 8.16 (2H, m).

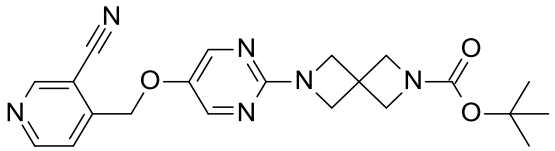
***N*-[*Trans*-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)cyclohexyl]-5-(tetrahydro-2H-pyran-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine 5b**



TFA (4 mL, 51.92 mmol) was added to a solution of *N*-(4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)cyclohexyl)-5-(tetrahydro-2H-pyran-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (370mg, 0.70 mmol) in DCM (6 mL) at 25 °C. The resulting mixture was stirred at room temperature for 4 hours, after which the solvent was removed under reduced pressure. NH_3/MeOH (7 M, 6.0 mL) was then added and the mixture stirred for another 16 h. The crude product was purified by preparative HPLC (Phenomenex Gemini-NX axia Prep C18 OBD column, 5µ silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of water (0.1% HCO_2H) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford the racemate (100 mg, 35.9 %) as a colourless solid. This was purified by preparative chiral-HPLC (Column: Chiralpak IA 2*25cm, 5 um; Mobile Phase A: Hexane, Mobile Phase B: EtOH; Flow rate: 15 mL/min; Gradient: 50 B to 50 B in 16 min; 254/220 nm; RT1: 9.56 [*cis*]; RT2: 12.52 [*trans*]). The fractions containing the desired *trans* stereoisomer were evaporated to dryness to afford the title compound (32 mg, 30%) as a colourless solid .

^{1}H NMR (500 MHz, DMSO-*d6*, 300 K) 0.89 - 1.07 (2H, m), 1.31 - 1.45 (2H, m), 1.55 (2H, qd, *J* 12.5, 4.2), 1.71 (2H, d, *J* 11.3), 1.78 - 1.91 (3H, m), 1.93 (2H, dt, *J* 13.9, 6.9), 3.22 (5H, s), 3.54 (2H, t, *J* 11.0), 3.90 (2H, dd, *J* 11.0, 3.1), 4.01 (1H, ddp, *J* 11.5, 8.0, 4.0), 4.59 (4H, s), 5.52 (1H, d, *J* 8.0), 6.83 (1H, d, *J* 1.9), 8.06 (1H, s), 11.29 (1H, s). ^{13}C NMR (126 MHz, DMSO-*d6*, 300 K) 28.2, 29.9, 31.9, 34.0, 37.8, 48.6, 61.3, 65.3, 66.8, 80.0, 101.0, 116.4, 119.1, 150.8, 151.1, 155.6. HRMS (ESI^+): Anal. calc. for $C_{22}H_{32}N_5O_2$ $(M+H)^+$: 398.2556; Found: 398.2574.

***Tert*-butyl 6-[5-[(3-cyano-4-pyridyl)methoxy]pyrimidin-2-yl]-2,6-diazaspiro[3.3]heptane-2-carboxylate 21b**



2-Bromo-5-((3-bromopyridin-4-yl)methoxy)pyrimidine (87 mg, 0.25 mmol), *tert*-butyl 2,6-diazaspiro[3.3]heptane-2-carboxylate (50 mg, 0.25 mmol) and potassium carbonate (112 mg, 0.8 mmol) were suspended in butyronitrile (1 mL) and heated to 150 °C for 3 hours in the microwave. The reaction mixture was then cooled to room temperature and diluted with EtOAc (75 mL), and washed sequentially with water (25 mL), and brine (25 mL). The organic layer was dried over Na_2SO4, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% MeOH in DCM. Fractions containing the desired product were evaporated to dryness to afford 404 mg of an inseparable mixture of the title compound and 2-bromo-5-((3-bromopyridin-4-yl)methoxy)pyrimidine as an orange powder. This was used in the next step without further purification.

m/z (ES^+) $(M+H)^+$ = 464.14.

Tert-butyl 6-(5-((3-bromopyridin-4-yl)methoxy)pyrimidin-2-yl)-2,6-diazaspiro[3.3]heptane-2-carboxylate (404 mg, 0.9 mmol), zinc cyanide (82 mg, 0.7 mmol), tris(dibenzylideneacetone)dipalladium(0) (32 mg, 0.03 mmol) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (40 mg, 0.07 mmol) were suspended in DMF (4 mL) and sealed into a microwave tube (evacuated and purged with nitrogen). The reaction was heated to 130 °C for 60 minutes in the microwave reactor, then cooled to room temperature and filtered through celite. The reaction mixture was diluted with EtOAc (100 mL) and washed sequentially with water (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO4, filtered and evaporated to afford crude product, which was purified by flash silica chromatography (40 g column), elution gradient 0 to 100% EtOAc in DCM, and then 0-10% MeOH in EtOAc. Pure fractions were evaporated to dryness to afford a yellow solid

(73 mg), which was further purified by preparative HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 50 mm diameter, 150 mm length), using decreasingly polar mixtures of water (containing 0.5% NH_3) and MeCN as eluents. Fractions containing the desired compound were combined and the pH adjusted to ~7 with 1 M HCl and 1 M $NaHCO_3$. Organic solvents were removed under reduced pressure to give a white suspension, wich was extracted with DCM (2 x 25 mLl) and the combined organics dried over Na_2SO4, filtered and evaporated to afford the title compound (20 mg, 20% over two steps) as a colourless solid.

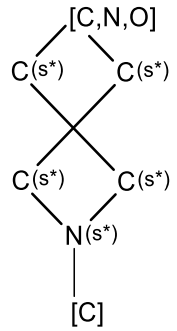
^{1}H NMR (400 MHz, DMSO-*d6*, 300 K) 1.37 (9H, s), 4.01 (4H, br s), 4.10 (4H, br s), 5.33 (2H, d, *J* 0.8), 7.69 - 7.79 (1H, m), 8.29 (2H, s), 8.87 (1H, d, *J* 5.1), 9.05 (1H, d, *J* 0.8). ^{13}C NMR (126 MHz, DMSO-*d6*, 300 K) 28.0, 32.4, 59.2, 60.3, 68.4, 78.6, 108.0, 115.4, 122.7, 145.4, 146.5, 148.8, 153.2, 153.5, 155.3, 159.3. HRMS (ESI$^+$): Anal. calc. for $C_{21}H_{25}N_6O_3$ $(M+H)^+$: 409.1988; Found: 409.1984.

Quantum Mechanics calculations of minimum energy conformations

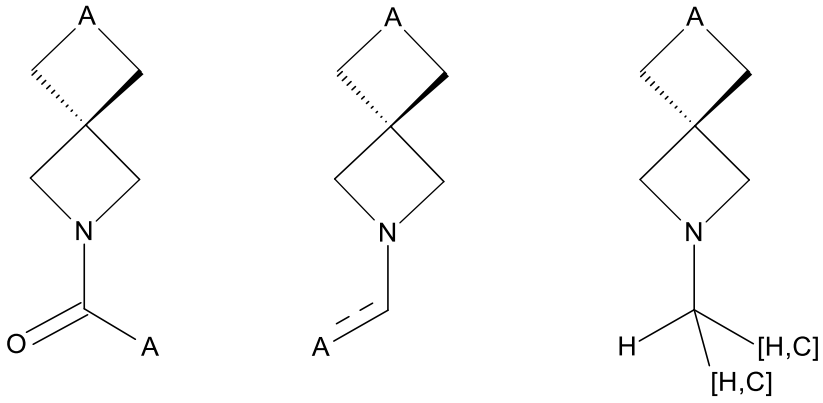
QM calculations were run using Jaguar, embedded within Schrodinger's Maestro package (version 2018-4).

Substructure query for matched molecular pair analyses

The following substructure was used to query our internal collection and identify the corresponding azaspiro[3.3]heptanes.



The corresponding morpholine, piperidine and piperazine matched pairs were then identified using a proprietary algorithm previously described[4] and the resulting list was manually curated to remove ambiguous matched pairs (mostly removing molecules with unknown chirality). Differences in measured log $D_{7.4}$ were calculated and pairs with at least one out of range value were removed. Matched pairs were then subdivided according to the following categories:



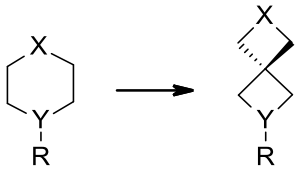
A **B** **C**

A. Amides and carbamates

B. Aromatic amines

C. Aliphatic amines

Statistical data for azaspiro[3.3]heptanes used as terminal groups.



R[a]	*Y*	*X*	*Δlog D*$_{7.4}$[b]	*StdDev*	*N*
	N	O	–0.17	0.36	9
	N	CH_2	+0.50	0.38	9
	N	NH	–0.61	0.58	2
	N	NAc	–0.15	0.33	4
	N	O	–0.44	0.28	16
	N	CH_2	+0.23	0.25	3
	N	NH	–0.58	0.38	4
	N	NMe	–1.12	0.22	9
	N	NAc	–0.80		1
	CH	NMe	–0.93	0.12	21
	CH	NAc	+0.12		1
	N	O	–0.75	0.26	10
	N	CH_2	+0.25	0.07	2
	N	NAc	–0.80		1

[a] Ar, R, R1 and R2 groups were identical across matched pairs, and no particular differentiation was made. [b] Difference in the octanol/water distribution coefficient at pH = 7.4 of the spiro analogue relative to the parent morpholine, piperazine or piperidine. Colour coding:

<−0.4	−0.4 to −0.2	−0.2 to +0.2	+0.2 to +0.4	>+0.4

Molecular Formula Strings

Entry	Smiles
1a	CC(=O)N1CCOCC1
1b	CC(=O)N1CC2(COC2)C1
2a	CN1CCN(CC1)C(=O)C
2b	CN1CC2(C1)CN(C2)C(=O)C
3a	CC(=O)N1CCN(CC1)C(=O)C
3b	CC(=O)N1CC2(CN(C2)C(=O)C)C1
4a	CC(=O)N1CCN(CC1)c2ccccc2
4b	CC(=O)N1CC2(C1)CN(C2)c3ccccc3
5a	c1[nH]c2ncnc(c2c1C3CCOCC3)N[C@H]4CC[C@@H](CC4)N5CCOCC5
5b	C1CC(CCO1)c2c[nH]c3ncnc(N[C@@H]4CC[C@H](CC4)N5CC6(COC6)C5)c23
6a	COc1ccc(cc1)c2oc(nn2)C(=O)N3CC(C3)Oc4ccc(CN5CCOCC5)cc4
6b	COc1ccc(cc1)c2nnc(o2)C(=O)N3CC(C3)Oc4ccc(cc4)CN5CC6(C5)COC6
7a	C(N1CCOCC1)c2ccc3OCOc3c2
7b	C(N1CC2(COC2)C1)c3ccc4OCOc4c3
8a	CC(=O)NC[C@H]1CN(C(=O)O1)c2ccc(N3CCOCC3)c(F)c2
8b	CC(=O)NC[C@H]1CN(C(=O)O1)c2ccc(N3CC4(COC4)C3)c(F)c2
9a	C(CN1CCOCC1)Oc2ccc(cc2)[C@@H]3CC[C@@]4(CC3)OO[C@@]5(O4)C6CC7CC(CC5C7)C6
9b	C(CN1CC2(COC2)C1)Oc3ccc(cc3)[C@@H]4CC[C@@]5(CC4)OO[C@@]6(O5)C7CC8CC(CC6C8)C7
10a	COc1cc2c(cc1OCCCN3CCOCC3)c(ncn2)Nc4ccc(c(c4)Cl)F

Entry	Smiles
10b	COc1cc2ncnc(Nc3ccc(F)c(Cl)c3)c2cc1OCCCN4CC5(COC5)C4
11a	c1cc(cc(c1)c2c[nH]c3c2c(ncn3)N4CCOCC4)C#N
11b	c1cc(cc(c1)c2c[nH]c3c2c(ncn3)N4CC5(C4)COC5)C#N
12a	CC(C)(C)CC(=O)Nc1c(nc(nc1OC)N2CCOCC2)OC
12b	CC(C)(C)CC(=O)Nc1c(nc(nc1OC)N2CC3(C2)COC3)OC
13a	c1ccc(cc1)S(=O)(=O)[C@@H]2C[C@H]([C@@H](C2)C(=O)N3CCOCC3)C(=O)NC4(CC4)C#N
13b	c1ccc(cc1)S(=O)(=O)[C@@H]2C[C@H]([C@@H](C2)C(=O)N3CC4(C3)COC4)C(=O)NC5(CC5)C#N
14a	CN1CCN(Cc2ccc3OCOc3c2)CC1
14b	CN1CC2(C1)CN(Cc3ccc4OCOc4c3)C2
15a	CC(=O)N1CCN(Cc2ccc3OCOc3c2)CC1
15b	CC(=O)N1CC2(C1)CN(C2)Cc3ccc4c(c3)OCO4
16a	CN1CCN(CC1)c2cc(Cl)cc3c2nc(N4CCNCC4)n3Cc5cc(C)c(F)c(C)c5
16b	Cc1cc(cc(c1F)C)Cn2c3cc(cc(c3nc2N4CC5(C4)CNC5)N6CCN(CC6)C)Cl
17a	Cc1cc(Cn2c(nc3c(cc(Cl)cc23)c4c(C)n[nH]c4C)N5CCNCC5)cc(C)c1F
17b	Cc1cc(cc(c1F)C)Cn2c3cc(cc(c3nc2N4CC5(C4)CNC5)c6c([nH]nc6C)C)Cl
18a	CSc1ccccc1Nc2c3c(c(ncn3)NCC4CC4)nc(n2)N5CCNCC5
18b	CSc1ccccc1Nc2nc(nc3c(NCC4CC4)ncnc23)N5CC6(CNC6)C5
19a	CN1C=C(C=C(Nc2ccc(cn2)N3CCN(CC3)C(=O)C)C1=O)c4ccnc(N5CCn6c7CC(C)(C)Cc7cc6C5=O)c4CO
19b	CN1C=C(C=C(Nc2ccc(cn2)N3CC4(CN(C4)C(=O)C)C3)C1=O)c5ccnc(N6CCn7c8CC(C)(C)Cc8cc7C6=O)c5CO
20a	Fc1ccc(CC2=NNC(=O)c3ccccc23)cc1C(=O)N4CCN(CC4)C(=O)C5CC5
20b	Fc1ccc(CC2=NNC(=O)c3ccccc23)cc1C(=O)N4CC5(CN(C5)C(=O)C6CC6)C4
21a	CC(C)(C)OC(=O)N1CCN(CC1)c2ncc(OCc3ccncc3C#N)cn2
21b	CC(C)(C)OC(=O)N1CC2(C1)CN(C2)c3ncc(cn3)OCc4ccncc4C#N
22a	Cn1c(nnc1SCCCN2CCN(CC2)c3ccccc3OC)c4ccccc4
22b	Cn1c(nnc1SCCCN2CC3(C2)CN(C3)c4ccccc4OC)c5ccccc5
23a	COc1ccccc1N2CCN(CC2)c3c4cnn(c4ncn3)c5ccccc5
23b	COc1ccccc1N2CC3(C2)CN(C3)c4c5cnn(c5ncn4)c6ccccc6
24a	c1cc(c(cc1F)N2CCN(CC2)c3ncc(s3)c4nnn(n4)CC(=O)O)Cl
24b	c1cc(c(cc1F)N2CC3(C2)CN(C3)c4ncc(s4)c5nnn(n5)CC(=O)O)Cl
25a	c1cc(cc(c1)Oc2ccc(cc2)Cl)CN3CCN(CC3)C(=O)Nc4cccnc4
25b	c1cc(cc(c1)Oc2ccc(cc2)Cl)CN3CC4(C3)CN(C4)C(=O)Nc5cccnc5
26a	c1ccc(cc1)SC[C@@H](CCN2CCOCC2)Nc3ccc(cc3S(=O)(=O)C(F)(F)F)S(=O)(=O)NC(=O)c4ccc(cc4)N5CCN(CC5)Cc6ccccc6c7ccc(cc7)Cl
26b	c1ccc(cc1)SC[C@@H](CCN2CCOCC2)Nc3ccc(cc3S(=O)(=O)C(F)(F)F)S(=O)(=O)NC(=O)c4ccc(cc4)N5CC6(C5)CN(C6)Cc7ccccc7c8ccc(cc8)Cl
27a	CN1CCC(CC1)S(=O)(=O)c2c(ccc(c2O)NC(=O)Nc3cccc(c3Cl)F)Cl
27b	CN1CC2(C1)CC(C2)S(=O)(=O)c3c(ccc(c3O)NC(=O)Nc4cccc(c4Cl)F)Cl
28a	Cc1cc(c2ccccc2n1)COc3ccc(cc3)S(=O)(=O)NC4(CCN(CC4)C(=O)C)C(=O)NO
28b	Cc1cc(c2ccccc2n1)COc3ccc(cc3)S(=O)(=O)NC4(CC5(C4)CN(C5)C(=O)C)C(=O)NO
29a	Oc1ccc(cc1)[C@H]2Sc3cc(O)ccc3O[C@H]2c4ccc(OCCN5CCCCC5)cc4
29b	Oc1ccc(cc1)[C@H]2Sc3cc(O)ccc3O[C@H]2c4ccc(OCCN5CC6(CCC6)C5)cc4
30a	NC(=O)C1=CN(c2ccc(F)cc2)c3ccc(CN4CCC(F)(F)CC4)cc3C1=O
30b	NC(=O)C1=CN(c2ccc(F)cc2)c3ccc(CN4CC5(C4)CC(F)(F)C5)cc3C1=O

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